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ORIGINAL ARTICLE

Detection of Specific IgE Antibodies in Sera of Japanese Birch-Allergic Patients Using Recombinant Allergens Bet v 1, Bet v 2 and Bet v 4

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ABSTRACT

Background: Birch pollen is the major allergen in pollinosis in northern Japan. IgE reactivity to individual birch pollen allergens has been shown to differ between populations of birch pollen-allergic patients living in different countries. In this study, we examined the IgE profiles to recombinant birch pollen allergens in birch-sensitive patients living in Sapporo.

Methods: This study used the sera of 40 patients with specific IgE toward birch pollen extract. Their sera were analyzed for specific IgE reactivity to individual birch pollen allergens (recombinant Bet v 1, Bet v 2 and Bet v 4) and natural birch pollen extract using Pharmacia CAP System™.

Results: Of 40 sera with positive CAP results for natural birch pollen extract, 39 (97.5%) had specific IgE towards Bet v 1; 6 (15%) contained specific IgE against Bet v 2. Bet v 4 reactivity was documented in only one subject (2.5%).

Conclusions: The present data suggest that the specific IgE reactivity profiles to birch pollen allergen in birch-sensitive patients in Sapporo correspond to those in Scandinavia, possibly due to the heavy birch pollen exposure in this area. This observation provides useful information for future birch allergen-specific immunotherapy in Japan.

KEY WORDS

Bet v 1, Bet v 2, birch pollinosis, RAST

INTRODUCTION

The major birch pollen allergen, Bet v 1, was first reported in 1989.[1] Other relevant birch pollen allergens are Bet v 2[2] and Bet v 4.[3,4] All these birch pollen allergens are commercially available as diagnostics. There have been some reports concerning the positive rate of the specific IgE against these birch allergens in Europe,[5,6] and IgE reactivity to individual birch pollen allergens has been shown to differ between populations of birch pollen-allergic patients living in different countries.[5] In the north of Japan, birch pollen is the major allergen in pollinosis. However, as yet, no author has presented an analysis of the IgE profiles to these birch allergens in Japan. In this study, we present an analysis of IgE profiles to recombinant birch pollen allergens for pollinosis patients living in northern Japan using the immunoenzymatic CAP method.

METHODS

This study used the sera of 40 patients with specific IgE toward birch pollen extract >0.70 kUA/l and the sera of 10 healthy control subjects without any symptoms of oral allergy syndrome (OAS) or allergic rhinitis. All patients were recruited from outpatients at the Department of Otolaryngology of Sapporo Medical University or Yamamoto ENT Clinic in the period between April and May 2006. All participating patients provided informed consent. 20 patients reported symptoms of OAS to pertinent fresh fruits. The other

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Table 1 Demographic characteristics of birch-allergic patients and control subjects

	Patients with OAS symptoms ($n = 20$)	Patients without OAS symptoms ($n = 20$)	Controls ($n = 10$)
Sex (male/female)	7/13	9/11	6/4
Age	39 (16–72)	45 (14–70)	36 (26–45)
Specific IgE to birch pollen (T3) (kU/L)	17.7 (1.4–94.4)	5.4 (0.9–25.2)	< 0.35

Data expressed as median values and range (in brackets).

Table 2 Percentage of birch-allergic patients with IgE reactivity to the recombinant allergens Bet v 1, Bet v 2, and Bet v 4

Recombinant allergens	Patients with OAS symptoms ($n = 20$) % (n)	Patients with out OAS symptoms ($n = 20$) % (n)	Controls ($n = 10$) % (n)
Bet v 1	100 (20)	95 (19)	0 (0)
Bet v 2	20 (4)	10 (2)	0 (0)
Bet v 4	0 (0)	5 (1)	0 (0)

20 patients had no OAS symptoms. Demographic and clinical characteristics of the patients and controls are summarized in Table 1.

IgE-specific antibodies for birch (t3), grass (g3), mugwort (w6), house dust mite (d1) and the recombinant birch pollen allergens (rBet v 1, rBet v 2 and rBet v 4) were evaluated using the immunoenzymatic CAP method (Pharmacia Diagnosis, Uppsala, Sweden) according to the manufacturer's instructions. The cutoff value of the assay was 0.35 kUA/l.

Spearman correlation coefficients were used to evaluate whether the serum levels of IgE to natural birch pollen extract and the IgE levels to recombinant Bet v 1 or Bet v 2 were correlated. Frequency analysis to evaluate specific IgE positivity to mite, grass and mugwort among Bet v 2 IgE positive or negative birch pollen-allergic patients was performed with Fisher's exact tests.

RESULTS

Of 40 sera with positive CAP results for natural birch pollen extract, 39 (97.5%) had specific IgE towards Bet v 1; 6 (15%) contained specific IgE against Bet v 2. Bet v 4 reactivity was documented in only one subject (2.5%) (Table 2). On the other hand, control subjects were negative for all of the birch-pollen allergens tested. There was no significant difference in IgE positivity to these birch allergens between patients with OAS and patients without OAS.

As shown in Figure 1, there was a strong correlation between the serum levels of IgE to natural birch pollen extract and the IgE levels to recombinant Bet v 1 ($r = 0.996$, $p = 1.02 \times 10^{-41}$). A weak, but significant, correlation was observed between the levels of IgE to birch pollen extract and IgE to Bet v 2 ($r = 0.536$, $p = 0.0036$) (Fig. 2). No statistical correlation was found between IgE to birch pollen extract and IgE to Bet v 4 ($r = -0.022$, $p = 0.676$).

In Bet v 2 IgE positive versus negative birch pollen-allergic patients, there was a significantly higher frequency of positive specific IgE antibodies to mite (6 of 6 *vs.*18 of 34, $p = 0.035$), grass (5 of 6 *vs.* 10 of 34, $p = 0.021$) and mugwort (5 of 6 *vs.* 5 of 34, $p = 0.0020$) (Table 3).

DISCUSSION

In the present study, we analyzed the IgE-reactivity profile among birch pollen-allergic patients in the northern part of Japan. There was a correlation between the serum levels of IgE to natural birch pollen extract and IgE levels to recombinant Bet v 1. While IgE antibodies to Bet v 1 were almost certainly present in patients from Sweden, Finland and Austria ($\geq$ 98) and common in France (90%), they were present in less than 70% of patients from Switzerland and northern Italy.[5] In contrast, IgE reactivity to Bet v 2 was rare in Scandinavian patients, while it was more abundant in patients living in central or southern Europe.[5,6] Thus, the present data suggest that the specificities of IgE reactivity profiles in relation to birch pollen allergen in Japanese birch-allergic patients correspond to those in Scandinavian patients, which differ from results obtained in patients from northern Italy.[5,6] Rossi *et al.* suggested that Bet v 2 reactivity in the patients from the northern Italy might indicate that these subjects have primarily been sensitized to allergens from other sources, such as grass pollen.[6] In Sapporo, only birch pollen concentrations are high,[7] suggesting that birch-allergic patients in Sapporo have primarily been sensitized to birch pollen allergen. To prove this hypothesis, a comparison of IgE profiles in the sera of birch pollen-sensitive patients in an area of lower exposure to birch pollen will be necessary.

Bet v 4 has been reported to be a calcium binding protein of birch pollen allergen.[4] Only 2.5% of patients with specific IgE towards birch pollen extract had serum IgE specific to Bet v 4 in the present study. This

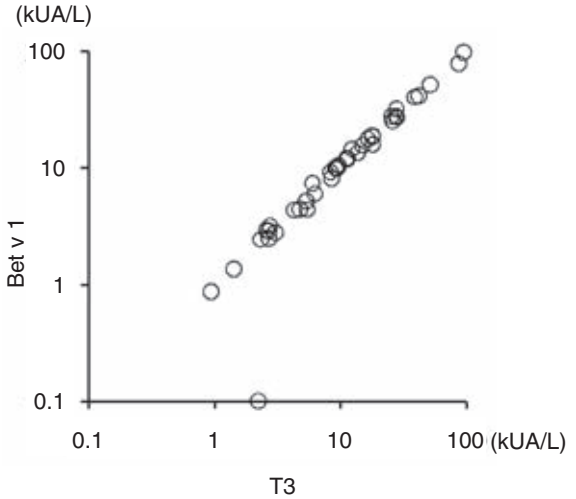

Fig. 1 Significant correlation between specific IgE levels to recombinant Bet v 1 and specific IgE levels to birch pollen extract (T3). ($p = 1.02 \times 10^{-41}$)

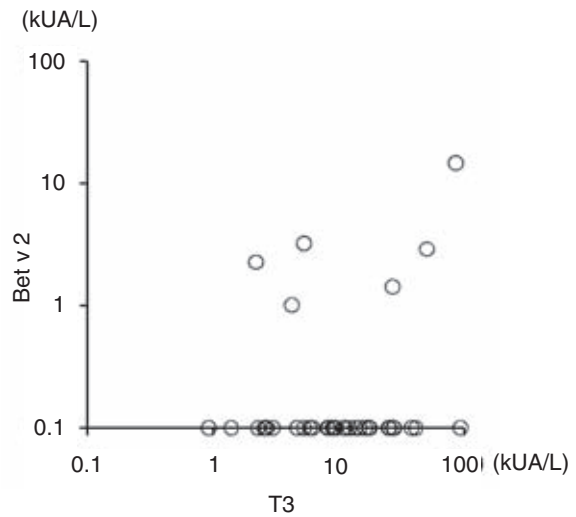

Fig. 2 A weak, but significant correlation between specific IgE levels to recombinant Bet v 2 and specific IgE levels to birch pollen extract (T3). ($p = 0.0036$)

observation is similar to that in a previous report in European populations regarding sensitivity to Bet v 4.[5,6] Moreover, there was no significant statistical correlation between IgE specificity to birch pollen extract and Bet v 4 in the present study. From these observations it appears that the clinical significance of Bet v 4 in birch-allergic patients may be minor.

In this study, we demonstrated that patients with IgE to Bet v 2 (profilin) frequently have IgE not only to other pollen but also to house dust mite. Although the frequency of birch pollen allergy in mugwort-allergic patients is high,[8] as yet, no author has reported a relationship between mite-allergy and birch-allergy. Profilins are present in almost all eukaryotic cells and are highly cross-reactive as panallergen.[9] Profilins are structurally conserved eukaryotic proteins which have been described as allergens in trees, grasses, weed pollens, fruits, vegetables, nuts, latex and even in man.[10] Profilin-sensitized patients may either exhibit clinically relevant reactivity to several of the allergen sources or be at risk of developing allergic symptoms.

Table 3 Percentage of birch-allergic patients with IgE reactivity to other major allergens

Allergen	Bet v 2 positive ($n = 6$) % (n)	Bet v 2 negative ($n = 34$) % (n)	p value
Mite	100 (6)	53 (18)	0.035
Grass	83 (5)	29 (10)	0.021
Mugwort	83 (5)	15 (5)	0.0020

The only treatment for IgE-mediated allergy that leads to long-lasting relief of symptoms is allergen-specific immunotherapy. According to the arguments outlined by Kazemi-Shirazi, patients with IgE antibodies specific for Bet v 1 are considered suited for birch pollen extract-based immunotherapy, and those patients with specific IgE to Bet v 2 and Bet v 4, but not to Bet v 1, are not suitable for birch pollen immunotherapy.[10] However, symptomatic medication is the most standard therapy for Japanese birch pollen allergic patients because allergen extract used for immunotherapy specific to birch pollen has not been permitted in Japan. Recently, the clinical efficacy of specific immunotherapy using Bet v 1 has been reported.[11,12] Our present study of IgE profiles will provide useful information for future birch allergen-specific immunotherapy in Japan.

In summary, we have shown that sensitization solely to Bet v 2 (profilin) is rare among birch pollen-allergic patients in northern Japan. The majority are sensitized to birch major allergen Bet v 1, possibly due to the heavy birch pollen exposure in this area.

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